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Anti-inflammatory effect of extract and fractions from the leaves of *Byrsonima intermedia* A. Juss. in rats

Lucimara Q. Moreira [a], Fabiana C. Vilela [b], Lidiane Orlandi [b], Danielle F. Dias [a], Ana Laura A. Santos [a], Marcelo A. da Silva [a], Renato Paiva [c], Geraldo Alves-da-Silva [a], Alexandre Giusti-Paiva [b,*]

[a] *Faculty of Pharmaceutical Sciences, Federal University of Alfenas, Alfenas – MG, Brazil*
[b] *Institute of Biomedical Sciences, Federal University of Alfenas, Alfenas – MG, Brazil*
[c] *Department of Biology, Federal University of Lavras, Lavras – MG, Brazil*

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ABSTRACT

Aim of the study: Byrsonima intermedia is commonly used for its antiseptic, antimicrobial, and anti-inflammatory properties in the treatment of diarrhea and dysentery in Brazilian folk medicine. The purpose of this study was to examine the anti-inflammatory activity of the aqueous extract and fractions of *Byrsonima intermedia* leaves.

Materials and methods: Rats with carrageenan-induced paw edema and fibrovascular tissue growth, which was induced by subcutaneous implantation of a cotton pellet, were used as acute and chronic animal models of inflammation to investigate the anti-inflammatory effects of the aqueous extract and the individual ethyl acetate (EtOAc) and aqueous fractions of *Byrsonima intermedia* and catechin. High-performance liquid chromatography (HPLC) was used to determine the fingerprint chromatogram of the aqueous extract and fractions of *Byrsonima intermedia*.

Results: The crude aqueous extract at test doses of 30–300 mg/kg p.o. clearly demonstrated anti-inflammatory effects by reducing carrageenan-induced paw edema, as did the ethyl acetate (100 mg/kg) and aqueous fractions (30–100 mg/kg). In the chronic inflammation rat animal model with fibrovascular tissue growth, the aqueous extract of *Byrsonima intermedia* (BiAE) at doses of 30–300 mg/kg and the individual EtOAc and aqueous fractions at doses of 30–100 mg/kg and catechin significantly reduced the formation of granulomatous tissue. The presence of catechin and phenolic compounds in the extract and fractions of *Byrsonima intermedia* was confirmed using HPLC.

Conclusion: BiAE and the individual EtOAc and aqueous fractions of *Byrsonima intermedia* exhibited chronic and acute anti-inflammatory efficacy in rats, which supports previous claims of its use in traditional medicine.

1. Introduction

The biodiversity of Brazilian is broad and several native Brazilian plant species have a long history of use in traditional medicine. These traditional medicinal plants contain compounds that may not only exhibit a broad range of therapeutic efficacy but also be useful in modern medicine. The Brazilian Cerrado (Neotropical Savanna) is one of the most biogeographically diverse regions of the world; it contains numerous native species of vascular plants (Blatt et al., 1998; Mendonça et al., 1998). Many of these plants are commonly used in traditional medicine for the treatment of several ailments (Blatt et al., 1998; Mendonça et al., 1998), including infectious and inflammatory diseases.

The genus *Byrsonima* belongs to the Malpighiaceae family of flowering plants, and it is widely distributed throughout tropical America. *Byrsonima intermedia* A. Juss. (*Byrsonima intermedia*), which is popularly known as "murici-pequeno," is native to the Brazilian Cerrado (Anderson, 1979; Nogueira et al., 2007; Sannomiya et al., 2007; Agra et al., 2008). *Byrsonima intermedia* leaves are traditionally used for the treatment of fever, tuberculosis, fungal and bacterial infections as well as dermal and gastrointestinal diseases (Nogueira et al., 2007; Agra et al., 2008). Previous studies have shown that the methanol extract of *Byrsonima intermedia* exhibits antimicrobial activity (Michelin et al., 2008; Pavan et al., 2009). The chemical constituents of the methanol extract of *Byrsonima intermedia* leaves has been shown to contain flavonoids, such as catechin, quercetin-3-*O*-α-arabinopyranoside,

* Corresponding author at: Instituto de Ciências Biomédicas, Universidade Federal de Alfenas-MG, 37130-000 - Alfenas – Minas Gerais, Brazil. Tel.: +55 35 3299 1303; fax: +55 35 3299 1063.
E-mail address: agiustipaiva@gmail.com (A. Giusti-Paiva).

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quercetin-3-*O*-β-galactopyranoside and amentoflavone (Sannomiya et al., 2007).

Based on the extensive use of *Byrsonima intermedia* in traditional medicine, the aim of this work was to evaluate the anti-inflammatory effects of the aqueous extract and fractions of *Byrsonima intermedia* leaves in acute and chronic inflammatory animal models.

2. Material and methods

2.1. Plant material

Byrsonima intermedia leaves were collected in November de 2009 in Ijaci (Minas Gerais, Brazil). Botanical identification was done in the Laboratory of Tissue Culture Plants of the Federal University of Lavras (Lavras, Minas Gerais, Brazil) by Dr. Renato Paiva. A voucher specimen of collected *Byrsonima intermedia* leaves was deposited at the herbarium of the Federal University of Lavras (voucher 17.601).

2.2. Preparation of the plant extracts and reference drugs

Byrsonima intermedia leaves were air dried at 40 °C and then ground into a powder. The powder was extracted with hot deionized water (100 °C) for 20 min. The aqueous extract was then lyophilized (45% yield) and stored under light protection at −5 °C. The lyophilized *Byrsonima intermedia* leaf extract was partitioned in succession with Ethyl acetate (EtOAc) and water, which yielded a dried EtOAc fraction (13.1% yield) and a water-soluble fraction. The water-soluble fraction residue was used to determine the bioactivity of *Byrsonima intermedia*. Doses of 30, 100, and 300 mg/kg of *Byrsonima intermedia* aqueous extract (BiAE); 10, 30, and 100 mg/kg of the EtOAc and aqueous fractions and 75 mg/kg catechin; and 0.2 mg/kg dexamethasone and 10 mg/kg indomethacin (as reference controls) were suspended in a 1% sodium carboxymethylcellulose (CMC) suspension in distilled water prior to being administered orally to rats (Vilela et al., 2010). Control animals were exposed to the same experimental conditions as the test groups, except the drug treatments were replaced with appropriate volumes of the CMC dosing vehicle (10 ml/kg).

2.3. Thin-layer chromatography fingerprints

Thin layer chromatography (TLC) of the lyophilized BiAE and the EtOAc and aqueous fractions was performed on 0.25 mm thick Merck Silica gel 60 F_{254}-precoated TLC plates, developed with $CHCl_3$/MeOH/H_2O (43:37:20, v/v/v) and visualized as previously described (Wagner and Bladt, 1996). Briefly, TLC plates were sprayed and developed with natural products-polyethylene glycol (NP/PEG) reagent, and the fluorescent flavonoids were detected under ultraviolet (UV) light with a 365 nm wavelength and then compared to catechin, rutin, and quercetin standards (1:1, w/v).

2.4. Analysis of the BiAE and the individual EtOAc and aqueous fractions using high performance liquid chromatography

High performance liquid chromatography (HPLC) analysis of the BiAE and the individual EtOAc and aqueous fractions were performed using a Shimadzu UFLC 20A using a Shimadzu CLC-ODS (250 – 4.6 mm) C18 column with a 5 μm particle size. Mobile phases were composed of a (A) 0.5 mM/l aqueous acetic acid and (B) 0.5% acetic acid in methanol. The gradient of the mobile phases (A:B) used for separation were a linear gradient 0–30 min (90:10 to 0:100) and 50 min (0:100) with a solvent flow rate of 1.0 ml/min, an injection volume of 25 μl at a concentration of 1 mg/ml. The eluent was detected with a photodiode array detector (DAD) with UV light at 268 nm. LC solution software (Shimadzu) was used for data collection.

2.5. Experimental procedures

2.5.1. Animals

Adult male Wistar rats weighing 180–220 g ($n = 8$ animals per group) were obtained from the Central Animal Facility of the Federal University of Alfenas (Alfenas, Minas Gerais, Brazil) and housed in a controlled 12 h light–dark room (lights on at 06:00 am) at 23 ± 1 °C with *ad libitum* access to water and food. The animals were habituated to the housing facilities for at least one week prior to the beginning of any experiments. All experiments were conducted in accordance with the Declaration of Helsinki on the welfare of experimental animals with the approval of the Ethics Committee of the Federal University of Alfenas (#221/2009).

2.5.2. Carrageenan-induced paw edema in rat

Rats were fasted overnight with free access to water, and then pedal inflammation was induced by treatment of the right hind paw with carrageenan as previously described (Vinegar et al., 1969; Vilela et al., 2010). Paw edema was measured with a plethysmometer (Model 7140, Ugo Basile, Italy). The basal volume of the right hind paw was determined prior to the administration of any drug. After the basal paw volume was measured, the animals were divided into experimental treatment groups ($n = 8$ per group) so that the mean basal paw volume of each group was similar. Vehicle, BiAE, EtOAc, aqueous fractions, catechin or indomethacin was administered orally 1 h prior to the intraplantar injection of 1 mg carrageenan in 100 μl. The paw volume was measured 1, 2, 3 and 4 h following the injection of carrageenan. Results are presented as the change of paw volume (ml) in relation to the basal paw volume.

2.5.3. Cotton pellet-induced granuloma tissue formation test

The effect of BiAE, the individual EtOAc and aqueous fractions or catechin on the chronic and proliferative phases of inflammation was assessed using a cotton pellet induced granuloma tissue formation rat model, as previously described by Swingle and Shideman (1972). Rats were anesthetized with tribromoethanol (250 mg/kg; i.p.), and then an autoclave sterilized cotton pellet that weighed 40 mg was implanted subcutaneously, one on each side of the abdomen, through a small ventral incision in the abdomen of the animal. After the implantation of the cotton pellet, each treatment group of rats ($n = 8$ per group) was administered vehicle, dexamethasone (0.2 mg/kg), BiAE (30–300 mg/kg), EtOAc fraction (10–100 mg/kg), aqueous fraction (10–100 mg/kg) or catechin (75 mg/kg) once daily for seven consecutive days. Eight days after implantation of the cotton pellet the animals were euthanized with halothane. Each implanted cotton pellet was removed with the surrounding fibrovascular tissue and dried at 40 °C for 24 h; afterward, the dry weight was measured. Results are expressed as the difference between the initial implanted cotton pellet weight and final dry mass of the cotton pellet and fibrovascular tissue.

2.6. Statistical analysis

All data was analyzed using GraphPad statistical software Version 4.0 and expressed as mean ± standard error of the mean (SEM) Statistically significant differences between treatment groups were calculated using analysis of variance (ANOVA) followed by a Dunnett's post hoc test. *p*-Values less than 0.05 ($p < 0.05$) were considered significant.

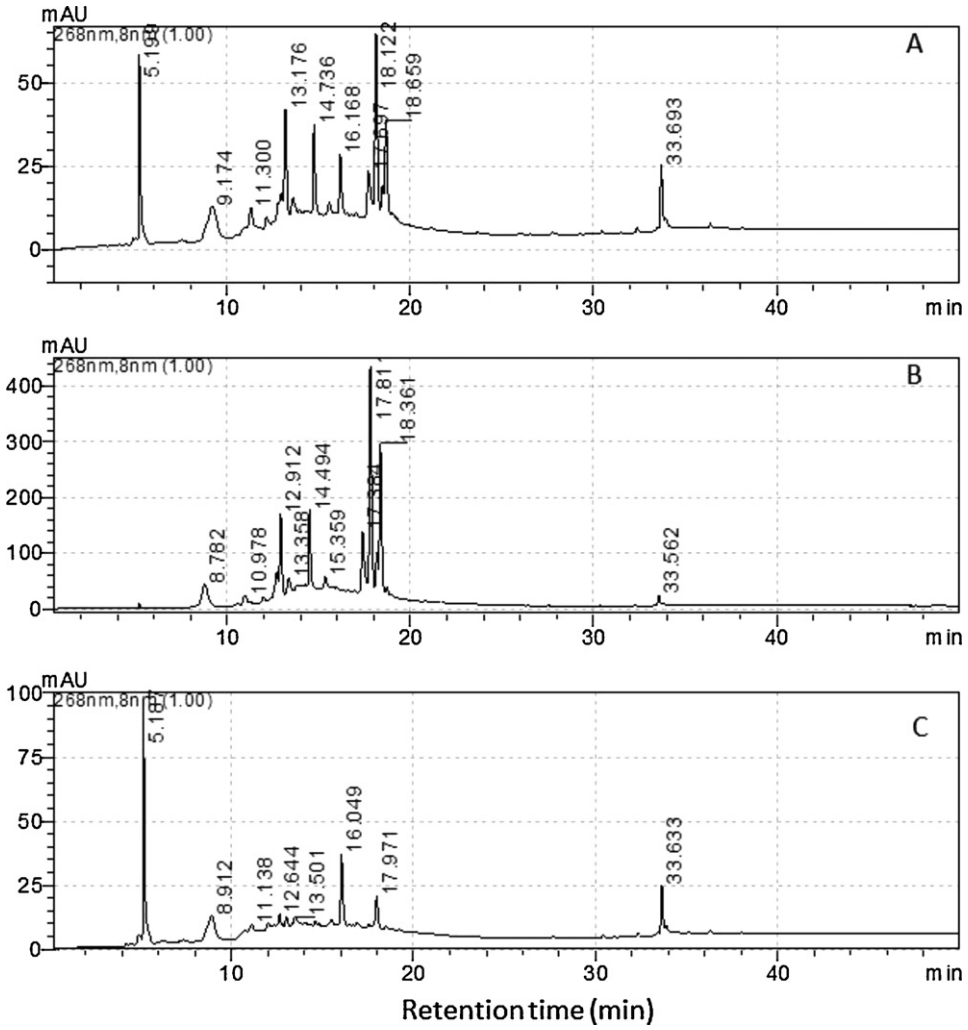

Fig. 1. HPLC chromatograms of the crude aqueous extract leaves of *Byrsonima intermedia* (A) and its ethyl acetate fraction (B) and aqueous fraction (C), which were monitored at 268 nm with a DAD.

3. Results

TLC fingerprints of each of the extracts from *Byrsonima intermedia* exhibited a specific chromatogram of catechin and flavonoids. The chromatogram of BiAE (Fig. 1A) and the EtOAc (Fig. 1B) and aqueous (Fig. 1C) fractions as well as a mixture of phenolic compounds that was composed of catechin and flavonoids were further examined using HPLC with a DAD with 268 nm wavelength. The presence of phenolic compounds in the BiAE and each of the individual EtOAc and aqueous fractions was confirmed by UV spectrometry (190–400 nm) and compared with standards. The HPLC chromatogram of BiAE had the peak at retention time (T_R) of 13.17 min was characterized for catechin and the peak at 11.30 min was catechin derivative that exhibited absorption maxima (λ_{max}) bands of decreasing intensity at 279 nm. The others peaks at 16.17 min, 17.69 min, 18.12 min and 18.66 min were exhibited UV absorption for some flavonoids derivatives. The EtOAc fraction had the catechin peak at 13.34 min with λ_{max} at 279 nm and two peaks of catechin derivatives at 10.98 min and 12.91 min. The peaks at 17.38 min, 17.81 and 18.36 min had exhibited UV absorption for flavonoid derivatives. Finally, the aqueous fraction had the peaks at 11.14 min and 13.50 min with UV absorption similar to catechin derivatives (λ_{max} at 279) and the peaks at 12.64 min, 16.05 min and 17.97 min with absorption like flavonoids derivatives. The catechin and derivatives content of BiAE and EtOAc and aqueous fractions were, respectively, 10%, 8% and 18%. The flavonoids derivatives content were 42% (BiAE), 44% (EtOAc fraction) and 40% (aqueous fraction).

The acute and chronic anti-inflammatory effects of each of the *Byrsonima intermedia* extract and fractions and catechin are shown in Figs. 2 and 3, respectively. Three hours after the injection of carrageenan, the oral administration of 30, 100 and 300 mg/kg BiAE reduced ($F_{4,32} = 7.77$; $p = 0.0002$; Fig. 2A) the edematous response by 41.8%, 87.3% and 86.7%, respectively. The reduction in inflammation by BiAE was comparable to a 10 mg/kg dose of indomethacin, which reduced edema inflammation by 90.0%. The EtOAc fraction ($F_{4,29} = 8.43$; $p = 0.00002$; Fig. 2B) had significant anti-inflammatory activity at a dose of 100 mg/kg and the aqueous fraction had significant anti-inflammatory activity at doses 30 and 100 mg/kg ($F_{4,24} = 9.925$; $p = 0.0003$ and $F_{4,30} = 17.1$; $p < 0.0001$, respectively; Fig. 2C). For catechin 75 mg/kg the anti-inflammatory effect was 24% reducing the edematous response ($F_{3,21} = 26.3$; $p < 0.0001$; Fig. 2D).

Fig. 3 shows the effects of BiAE and its fractions and catechin on granulomatous tissue growth. Oral administration of BiAE at doses of 30, 100 and 300 mg/kg produced a significant reduction in the formation of granulomatous tissue on the implanted cotton pellets ($F_{4,32} = 11.75$; $p < 0.0001$; Fig. 3A) when compared to vehicle control. A significant decrease in granulomatous tissue growth was observed following oral administration of all doses

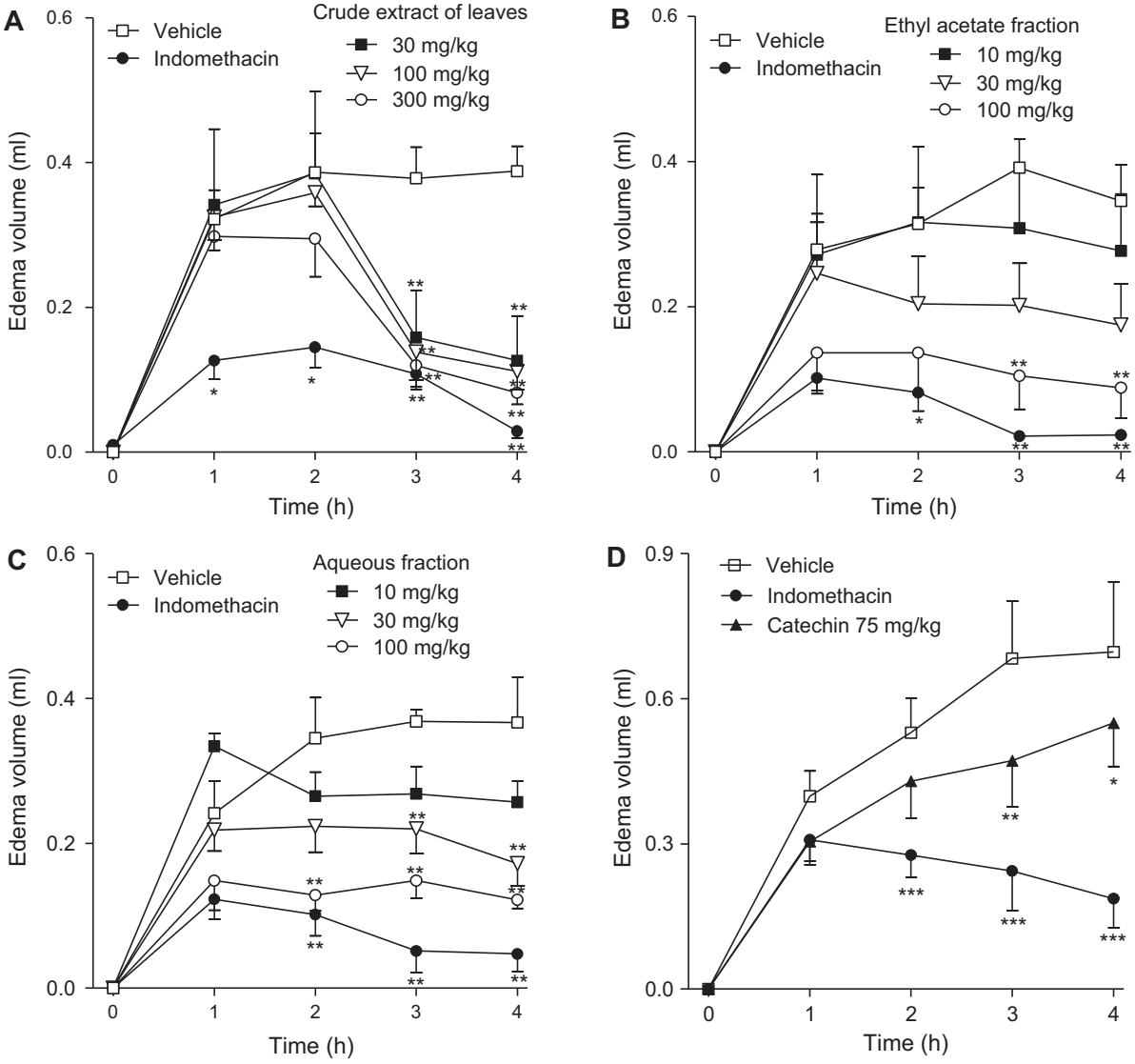

Fig. 2. Evaluation of the anti-inflammatory efficacy of the crude aqueous extract leaves of *Byrsonima intermedia* (A) and its ethyl acetate fraction (B) and aqueous fraction (C) and catechin (D) in a carrageenan induced paw edema rat model. The asterisks denotes the level of significance when compared to the control group: $^{*}p < 0.05$; $^{**}p < 0.01$.

of the EtOAc ($F_{4,26} = 11.49$; $p < 0.0001$; Fig. 3B) and aqueous fractions ($F_{4,28} = 11.84$; $p < 0.0001$; Fig. 3C). The effect of BiAE and the individual EtOAc and aqueous fractions was comparable to the reduction of the formation of granulomatous tissue following the administration of dexamethasone. Animals that were treated with catechin showed a reduction of granulomatous tissue growth of 26% when compared to vehicle control ($F_{3,20} = 31.5$; $p < 0.0001$; Fig. 3D).

4. Discussion

Anti-inflammatory compounds function by inhibiting specific enzymes and/or by acting as an antagonist of specific receptors and the translational response of mediators that are involved in an inflammatory response. Widely used cyclooxygenase (COX) inhibitors (Chan and Carter, 2010) and corticosteroids function by blocking the production and/or action of several pro-inflammatory mediators (Serhan, 2008). Corticosteroids are effective in the treatment of inflammatory diseases, but their long-term use may require increased doses and cause unwanted side-effects. It has been shown that prolonged treatment with non-selective COX inhibitors may cause gastrointestinal bleeding or kidney damage, and the more selective COX-2 inhibitors may cause an increase in the risk of development of cardiovascular disease (Mukherjee et al., 2001). Therefore, there is an urgent need to identify effective new anti-inflammatory therapies that have less possibility of aversive side effects (Barkin et al., 2010; Brueggemann et al., 2010; Capone et al., 2010).

Natural compounds that are derived from many plants and exhibit various anti-inflammatory mechanisms of action can be used to treat inflammatory diseases (Calixto et al., 2003; Rios et al., 2009). Many of the plants that are used in traditional medicine exhibit pharmacological properties and may offer significant potential as therapeutic agents. Tea made from small-murici has been popularly used in diarrhea and dysentery as well as for antifungal and anti-inflammatory activity (Rodrigues and Carvalho, 2001; Orlandi et al., 2011).

The present study examined the anti-inflammatory properties of the aqueous extract and the individual EtOAc and aqueous fractions of *Byrsonima intermedia* leaves.

Carrageenan-induced paw edema is an acute inflammatory animal model that is effective in studying orally active anti-inflammatory drugs (Winter et al., 1962; Morris, 2003). The development of carrageenan-induced edema is commonly

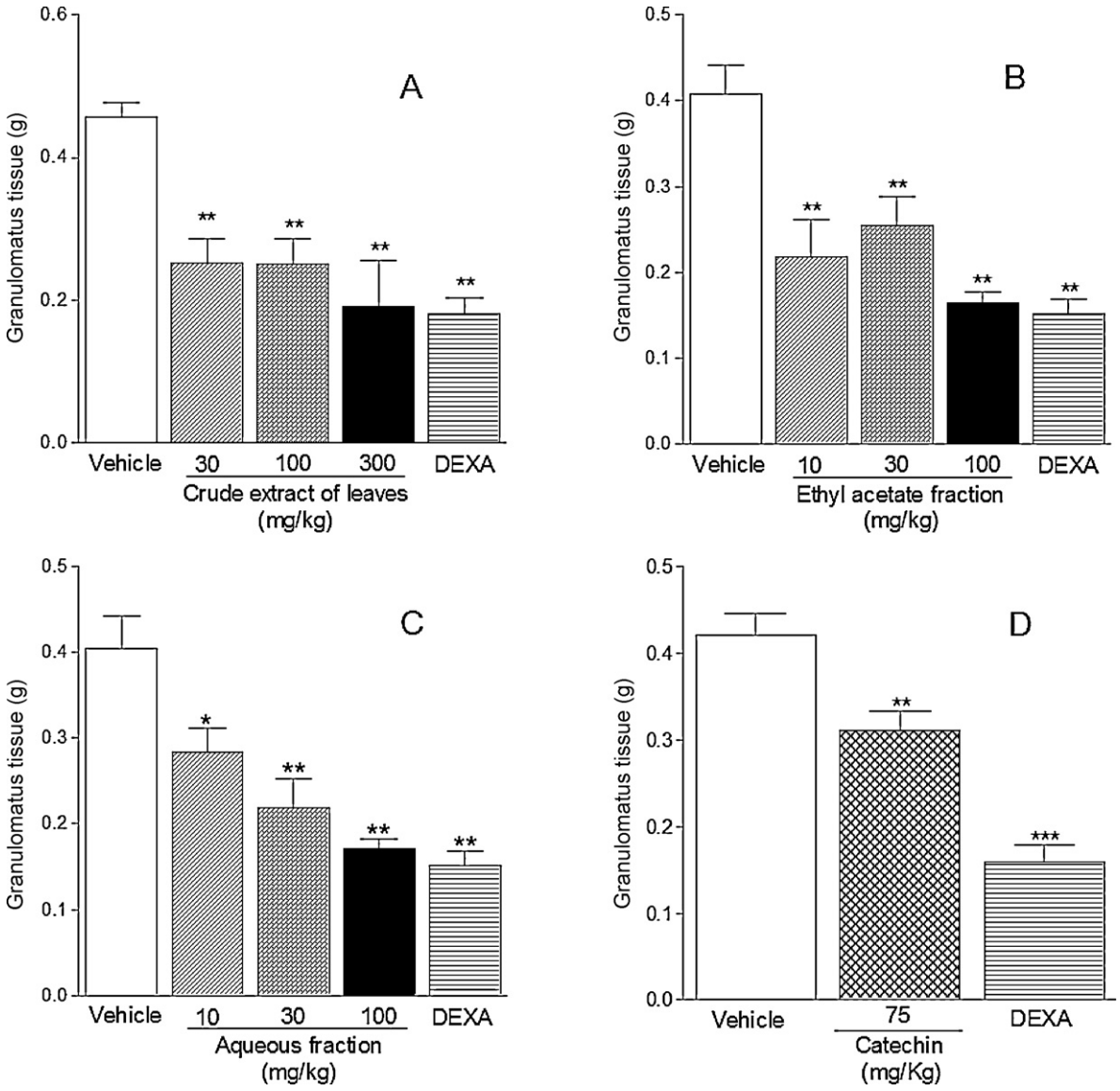

Fig. 3. The anti-inflammatory efficacy of the crude aqueous extract leaves of *Byrsonima intermedia* (A) and its ethyl acetate fraction (B) and aqueous fraction (C) and catechin (D) in the reduction of granulomatous tissue formation. The asterisks denote the level of significance when compared to the control group: $^{*}p < 0.05$; $^{**}p < 0.01$.

distinguished by the presence of the early exudative stage of inflammation, which is one of the most important characteristics of inflammatory pathology (Morris, 2003). Carrageenan-induced edema caused the release of several inflammatory mediators; the initial phase of inflammation includes the release of bradykinin, histamine and serotonin by cells near the carrageenan injection point. Several hours after treatment with carrageenan, there is an increase in the release of prostaglandins by macrophages, which are mediated by bradykinin and leukotrienes (Seibert et al., 1994; Ueno and Oh-ishi, 2002; Passos et al., 2004).

The induction of granulomas by subcutaneous insertion of a cotton pellet into a rat is widely used to study the transudative and proliferative phases of chronic inflammation. In this study, the aqueous extract and the EtOAc and aqueous fractions obtained from *Byrsonima intermedia* leaves and catequin decreased the wet and dry weight of the cotton pellets compared to control groups. This reduction in inflammatory granuloma tissue may be due to both the ability of *Byrsonima intermedia* to reduce the number of fibroblasts and the synthesis of collagen and mucopolysaccharides, which are natural proliferative agents involved in the formation of granuloma tissue.

The TLC and HPLC analysis of BiAE and the individual EtOAc and aqueous fractions showed that they contained phenolic compounds, which were further identified as catechin and flavonoids. The anti-inflammatory mechanism of action of phenols is caused by a reduction of NF-κB transcription by the inhibition of IκB kinase activity and the reduction of the expression of several target genes that are indispensable to the inflammatory process (Yang et al., 1998; Pan et al., 2000; Ichikawa et al., 2004). Catechins inhibit the adhesion and migration of neutrophils through endothelial cell monolayers by several mechanisms, which include the suppression of the production of chemokines at the site of inflammation (Hofbauer et al., 1999; Takano et al., 2004), reduced expression of cytokine-induced vascular cell adhesion protein 1 (VCAM-1) in endothelial cells and the reduction of monocyte adhesion to the endothelial monolayer, which is independent of NF-κB activation (Ludwig et al., 2004). Earlier studies showed that catechin inhibits secondary inflammatory reactions in rats with chronic inflammation. Catechin modulates the production of proinflammatory cytokines and reduced the level of PGE2 in rats (Tang et al., 2007).

The anti-inflammatory properties that were observed in this study may be due to the pharmacological activity of one or a

combination of several of the compounds present in *Byrsonima intermedia*, since the BiAE and fractions exercised greater anti-inflammatory activity compared to that catechin could be due to the synergistic effect. Reports have shown that crude plant extracts are more active pharmacologically than their isolated active principles (Hamburger and Hostettmann, 1991). This may be due to the synergistic effects of the various components present in the extracts. Other studies report these effects of phytochemicals including catechin and derivatives with various other phytochemicals (HemaIswarya and Doble, 2006).

In conclusions, the results presented in this study suggests that the aqueous extract that was obtained from *Byrsonima intermedia* leaves exhibited potent anti-inflammatory activity in acute and chronic inflammatory animal models. The precise mechanisms that are involved in the production of the anti-inflammatory response of *Byrsonima intermedia* extracts are not completely understood, but they may be caused by the presence of catechin and flavonoids in the aqueous extract of *Byrsonima intermedia* leaves. These results confirm the possible use of *Byrsonima intermedia* in traditional medicine as a therapeutic agent for the treatment of inflammatory diseases.

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